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Original Article

Brain-derived neurotrophic factor protein and mRNA levels in patients with bipolar mania – A preliminary study

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ABSTRACT

Background: Brain-derived neurotrophic factor (BDNF) protein or mRNA levels may be involved in the pathophysiology of bipolar disorder. However, the results were inconsistent. We aimed to simultaneously investigate the relationship of BDNF protein and mRNA levels in peripheral blood of patients with bipolar mania.

Methods: Patients with bipolar mania (n = 30) and healthy controls (n = 30) were recruited during our one-year study. Psychiatric diagnoses were made according to *Diagnostic and Statistical Manual of Mental Disorders, 4th Edition* criteria. The scores of the Young Mania Rating Scale (YMRS) of patients with bipolar mania were greater than 26. All participants had peripheral blood drawn to analyze the serum BDNF protein and mRNA levels.

Results: Using *t*-test, patients with bipolar mania had a lower BDNF protein and mRNA levels than did the healthy controls ($p < 0.001$ and 0.049, respectively), however, the statistical significances were lost after analysis of co-variance adjusted for age and body mass index. Twenty seven out of 30 patients with bipolar mania remained in the study after the 4 weeks of mood stabilizer treatment. Patients' BDNF protein and mRNA levels did not change significantly after 4-week treatment.

Conclusions: Our study found that serum BDNF protein and mRNA levels in patients with bipolar mania were lower than healthy controls, but a larger sample size will be needed to confirm this finding.

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At a glance commentary

Scientific background

Brain-derived neurotrophic factor (BDNF) has been suggested as a molecular candidate for developing bipolar disorder. Blood BDNF protein levels and its relationship with various mood states of bipolar disorder had been extensively studied, but studies regarding BDNF mRNA in bipolar disorder were scarce.

What this study adds to the field

We aimed to simultaneously investigate the relationship of BDNF protein and mRNA levels in peripheral blood of patients with bipolar mania. Further investigation with a larger sample size would better reveal the relationship between BDNF protein and mRNA levels with the pathophysiology of bipolar mania.

Several studies have adopted the brain-derived neurotrophic factor (BDNF) as a molecular candidate for developing bipolar disorder [1–8]. BDNF can cross the blood–brain barrier in both directions [9,10]. Blood BDNF levels correlate positively with cortical BDNF levels in mice, rats, and pigs [9,11]. These findings suggest that BDNF measured in blood could reflect illness activity in the brain.

Bipolar disorder is a unique psychiatric disease which features three distinct mood states: manic, depressive, and euthymic states. Blood BDNF protein levels and its relationship with various mood states of bipolar disorder had been extensively studied [12,13], though not all studies are consistent [14]. Studies regarding BDNF mRNA in bipolar disorder were scarce and did not separate the different mood states in the data analysis [15,16]. The manic state of bipolar disorder is usually managed by both mood stabilizers and antipsychotics, and earlier studies found an increase in BDNF levels following the treatment for acute mania [12].

Overall, the data of BDNF protein levels in bipolar disorder had been inconsistent, and the data of BDNF mRNA levels in bipolar mania had been scarce, this study aimed to simultaneously investigate BDNF protein and mRNA levels in the peripheral blood of patients with bipolar mania, before and after 4-week treatment, compared to healthy subjects.

Methods

Subjects

From November 2012 to July 2015, patients diagnosed with bipolar mania were evaluated according to the DSM-IV criteria using a semi-structured interview [17]. Patients with bipolar mania who were unmedicated for a period of at least 2 weeks and scored over 26 on the Young Mania Rating Scale (YMRS) [18] were recruited for the study. Healthy controls were evaluated with the Chinese Health Questionnaire-12 [19]. No participants had systemic diseases, such as cardiovascular disease, liver disease, or thyroid disease. The patients were excluded if they were heavy smokers, alcohol dependent, or illicit substance abusers. All experiments were performed in accordance with relevant guidelines and regulations. Approval was obtained from the ethics committee of the Institutional Review Board of Chang Gung Memorial Hospital. All patients and the healthy controls had the ability to provide written informed consent. All assessments were conducted by the same senior psychiatrist. The severity of YMRS was assessed again after a 4-week treatment.

Mood stabilizers consisting of either valproate (600–1500 mg/d) or lithium (900–1200 mg/d) were administered during the hospitalization. Some patients' treatment regimens also include other psychotropic drugs, including risperidone (1–4 mg/d), olanzapine (5–15 mg/d), lorazepam (1–3 mg/d), or hypnotics (i.e., zolpidem, 10–20 mg/d). 15 mL of venous blood was obtained from each participant. Another 15 mL of venous blood were drawn again from patients with bipolar mania after a 4-week treatment. Blood samples were collected in the morning at 7:00 a.m. after the patients fasted for 8 h.

Serum BDNF protein levels

Serum BDNF protein levels were measured using a commercially available ELISA kit of the sandwich type (BDNF Emax Immunoassay System; Promega; USA). Each system contained anti-BDNF mAb, Block&Sample 5X buffer, BDNF standard, antihuman BDNF pAb, anti-IgY HRP, TMB solution, peroxidase substrate, and protocol. All samples were assayed or duplicated by the same senior technologist.

mRNA isolation and reverse transcription polymerase chain reaction

Peripheral blood (2.5 mL) was collected using a PAXgene Blood RNA Tube (Qiagen) and extracted using a PAXgene Blood RNA Kit. The total RNA (1 μg) was reverse-transcribed into cDNA by using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). The PCR of BDNF and β-actin gene expression (primer pairs ordered from Promega Biosciences) were conducted using SYBR Green (Applied Biosystems), using 5 μL of cDNA in a 20-μL final volume and 0.5 μM of each primer (final concentration). Quantitative PCR was performed using a 7500 Fast Real-Time PCR System (Applied Biosystems) for 45 cycles at 95 °C for 5 s, at a specific annealing temperature of 60 °C for 5 s and 72 °C for 12 s. Amplification specificity was investigated using the melting curve following the manufacturer instructions. The results were analyzed using 7500 Fast Real-Time PCR System Software v1.4.1 (Applied Biosystems). Gene expression levels were expressed as the concentration ratios between PCR products and β-actin in the same sample.

Data analysis

All results were expressed as mean ± standard deviation (SD). The statistical differences of BDNF protein levels and mRNA levels between patients with bipolar mania and healthy controls were determined by *t*-tests and analysis of covariance (ANCOVA) with age and body mass index (BMI) adjustment. The statistical differences of BDNF protein levels and mRNA

levels of patients of bipolar mania before and after treatment were determined by paired *t*-tests. *Pearson* correlation was performed between BDNF protein levels, BDNF mRNA levels, YMRS score, and illness duration. A *p* value less than 0.05 was considered to be statistically significant.

Results

During this period, 30 patients with bipolar mania and 30 healthy controls were recruited for this study. Table 1 lists the demographic data, and serum BDNF protein and mRNA levels at baseline in all participants. 27 patients with bipolar mania remained in the study after the 4-week treatment.

Using t test, patients with bipolar mania had a lower BDNF protein level than did the healthy controls ($t = 6.361$, $p < 0.001$) [Fig. 1]. However, no significant difference existed after an ANCOVA with age and BMI adjustment ($F = 0.080$, $p = 0.778$). After the 4-week treatment with mood stabilizers, the patients' BNDF protein level did not change significantly from the baseline ($t = -0.753$, $p = 0.458$).

The results of a t test revealed that the patients with bipolar mania had lower BDNF mRNA levels than did the healthy controls ($t = 2.013$, $p = 0.049$) [Fig. 2]. However, no significant difference was observed based on an ANCOVA with age and BMI adjustment ($F = 0.021$, $p = 0.885$). After the 4-week treatment with mood stabilizers, the patients' BNDF mRNA level appeared to increase from the baseline, but did not reach statistical significance ($t = -1.578$, $p = 0.127$).

Pearson correlation revealed no significant correlations between BDNF protein levels, BDNF mRNA levels, YMRS score, and illness duration.

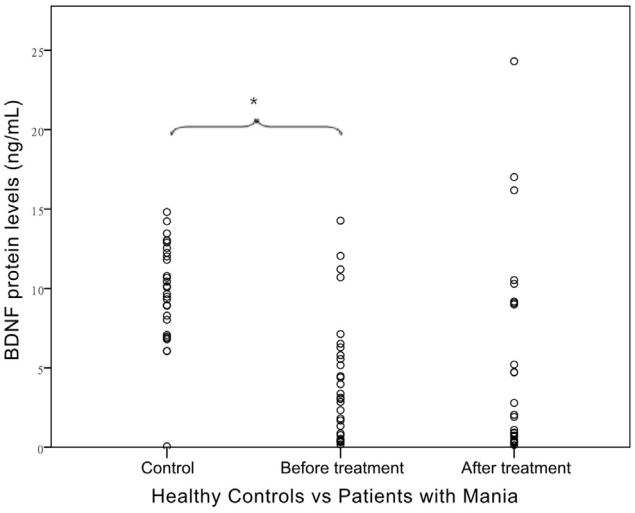

Fig. 1 – Expression of BDNF protein levels in controls and bipolar disorder. *: $p < 0.05$.

Discussion

In this study, we found that BDNF mRNA levels are lower in the patients of bipolar mania in the t test, however, no statistical significance was found after ANCOVA adjusted with age and BMI. Most earlier studies concerning BDNF mRNA and bipolar disorder utilized postmortem brains. BDNF mRNA levels were lower in bipolar group as compared to the control group [20,21]. BDNF mRNA levels were also decreased in layer VI of inferior temporal gyrus in layer V and/or VI of superior temporal gyrus [16] and cornu ammonis subfield 4 [22] in bipolar disorder. A study focused on pediatric patients found BDNF mRNA levels in lymphocytes were lower in bipolar disorder patients compared to healthy controls [15]. The bipolar disorder patients from aforementioned studies included patients in both manic and depressive episode. The strength of our study is the selectivity of recruiting manic patients only.

Our other finding is that serum BDNF protein levels are lower in the patients of bipolar mania in the *t* test, however, no statistical significance was found after ANCOVA adjusted with age and BMI. This finding is in line with most earlier studies. A recent meta-analysis of six depressive, eight manic, and nine euthymic state studies showed that BDNF levels were decreased in patients with an acute depressive episode and an acute manic episode, and no significant differences were detected in BDNF levels of euthymic patients when compared with control subjects [13]. An earlier meta-analysis of thirteen studies found similar results [12]. However, our earlier investigation found no significant differences in serum BDNF protein levels between patients with bipolar mania and healthy controls [14]. This discrepancy could be caused by the small sample size of our previous investigation. Our finding in

Table 1 – Demographic data and serum BDNF protein and mRNA levels of patients with bipolar mania and controls.

Diagnostic groups	Age	Education (years)	Duration of illness (years)	BDNF protein levels (ng/ml)	BDNF mRNA levels	YMRS score
Healthy controls (n = 30)	30.2 ± 5.1	17.4 ± 1.7	–	9.8 ± 3.1	2.2 ± 2.9	
Men (n = 16)	30.0 ± 5.0	17.9 ± 1.8	–	8.8 ± 3.3	1.4 ± 1.8	
Women (n = 14)	30.5 ± 5.4	16.8 ± 1.3	–	11.0 ± 2.4	3.0 ± 3.7	
Bipolar mania (n = 30)	39.3 ± 11.3	12.2 ± 1.7	14.0 ± 10.4	4.0 ± 3.9	0.9 ± 0.8	35.3 ± 6.8
Men (n = 14)	43.0 ± 10.9	12.4 ± 0.8	18.8 ± 7.8	4.2 ± 4.3	0.8 ± 1.0	36.2 ± 6.9
Women (n = 16)	36.1 ± 11.0	12.1 ± 2.3	9.9 ± 10.9	3.9 ± 3.6	1.0 ± 0.5	34.5 ± 6.8
After treatment (n = 27)				5.0 ± 6.3	1.4 ± 2.0	4.7 ± 7.4
Men (n = 13)				4.5 ± 7.7	1.3 ± 1.8	5.4 ± 10.1
Women (n = 14)				5.4 ± 4.9	1.5 ± 2.2	4.0 ± 7.2

BDNF = brain-derived neurotrophic factor; YMRS = Young Mania Rating Scale.

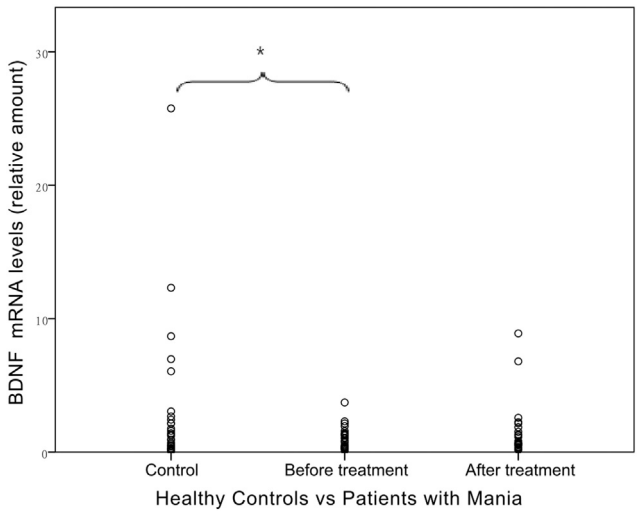

Fig. 2 – Expression of BDNF mRNA levels in controls and bipolar disorder. *: $p < 0.05$.

this study confirmed that serum BDNF protein levels were decreased in patients in an acute manic state.

We found no significant increase of BDNF protein and mRNA levels after four weeks of mood stabilizer treatment. A meta-analysis of thirteen studies found an increase in BDNF levels following the treatment for acute mania [12]. After an average of 52 days of treatment, the initially decreased BDNF levels of acute manic patients showed a sharp increase [23]. In another study, after 8 weeks of treatment, pediatric bipolar patients had significantly higher BDNF mRNA levels [15]. In contrast, our earlier work also found no significant change in serum BDNF protein levels after a 4-week treatment with mood stabilizers [14]. A possible explanation could be our shorter follow-up time, because in this study, BDNF mRNA levels did show a trend of increase after treatment but did not reach statistical significance. Another explanation is the frequent co-prescription of antipsychotics for treating bipolar mania. Different antipsychotics may have different effect on BDNF protein and mRNA levels. In rats, haloperidol decreased BDNF protein and mRNA levels, while olanzapine increased BDNF protein and mRNA levels [21]. Patients treated with antipsychotics and/or lithium had lower serum BDNF levels while patients treated with valproate and/or antidepressants had higher serum BDNF levels [24]. After a 16 week follow-up, extended-release quetiapine increases BDNF levels with time in those with a depressive episode, but decreases BDNF levels with time in those with a manic/mixed episode [25]. The frequent co-prescription of antipsychotics in addition to mood stabilizer to treat acute manic patients made the change of BDNF protein and mRNA levels after treatment difficult to interpret.

The small sample size is one limitation of this study. The prescription of antipsychotics in addition to mood stabilizers also made the interpretation difficult. The shorter follow-up period, compared to other studies, could also cause differences in the findings. A randomized controlled study with a longer follow-up period and larger sample size could further investigate these questions.

Conclusion

Our study found that serum BDNF protein and mRNA levels in patients with bipolar mania were lower than healthy controls, but a larger sample size will be needed to confirm this finding.

Conflicts of interest

The authors declare that they have no competing interests.

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